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Adding Specificity to Artificial Transcription Activators

In this issue, Mapp and colleagues describe a significant advance in the design of artificial transcription activators that function in a cell-type-specific manner. [1] The authors show that peptides selected for binding a component of the yeast transcription complex require its presence for effective transcriptional activation.

An overriding goal for chemical biologists is to develop tools for decoding the complicated networks of protein-protein interactions that execute the genetic program of an organism. One intricate and incompletely understood cellular process involves the initiation of transcription that ultimately leads to the transfer of information from DNA into RNA. Transcriptional activators that govern the expression of a specified gene are minimally composed of two modules: a sequence-specific DNA binding domain (DBD) that finds the promoter region of interest, and an activation domain (AD) that recruits the appropriate cellular machinery to the promoter via protein-protein interactions (Figure 1) [2].

Chemists are aiming to develop synthetic transcription activators (and repressors) that can selectively modulate the expression of any gene of interest [3]. Recent research efforts have afforded several ligands for sequence-specific binding of target DNA. These designed DBDs include pyrrole-imidazole polyamides [4], peptide nucleic acids [5], triplex-forming oligonucleotides [6], and zinc finger proteins [7]. While the DNA binding properties of these molecules have been characterized in detail, the precise role of the ADs in transcription has not been as clearly defined. The ADs are involved in the recruitment of coactivators, chromatin-modifying enzymes, and other components of the transcriptional machinery. Although several ADs may bind to a common target in transcriptional machinery, unlike DBDs they do not always share defined structural motifs [2].

Activation domains comprised of multiple acidic and hydrophobic residues ("acidic activators") function effectively in eukaryotes. An important feature of these strong ADs may be their ability to interact with multiple targets in the transcription complex [2]. But if this promiscuity is a necessary qualification for a strong AD, it may be difficult to build ADs that are cell-type- and species-specific. Thus, a key question for the development of next generation of transcription activators is whether *potent* activation can be achieved by specifically targeting individual components of the transcriptional machinery. Several research groups have now begun addressing this fundamental issue by creating ligands for distinct proteins found in the transcription complex. Montminy and Kodadek utilized phage display to isolate peptide ligands for p300/CREB binding protein (a histone acetyltransferase) and yeast repressor (Gal80), respectively, and have shown that these peptides are strong activators of transcription [8, 9]. Schepartz and coworkers have explored miniature protein scaffolds that display α-helical motifs to isolate high-affinity ligands for the CREB binding protein (CBP) [10]. The isolated miniprotein afforded a potent activator of p300/CBP-dependent transcription when fused to a Gal4 DBD [11]. Uesugi and coworkers used a small-molecule library to isolate ligands that mimic the AD of ESX (an epithelial-specific transcription factor) [12, 13]. Subsequently, these small-molecule ADs were fused to a sequence-specific DNA binding domain derived from pyrrole-imidazole polyamides to create synthetic transcription factors [14]. The Uesugi approach shows that it is possible to generate small-molecule activators by identifying inhibitors of the transcription factor-target protein interactions. Mapp and coworkers successfully showed that small-molecule transcription factors may also be constructed by incorporating key functional groups from acidic activators into isoxazolidine-based scaffolds [15]. This strategy elegantly translates the amphipathic nature of the acidic activators into the small-molecule regime.

Mapp's group is also simultaneously pursuing an alternative method for the development of potent activators [16]. This approach involves screening of synthetic peptide libraries to isolate short ADs that target specific components of the transcription machinery. In the reported case, peptides that target Gal11 (Med15), a component in yeast mediator complex, were isolated. A key finding in this paper was that the binding affinities of the peptides for Gal11 may not be the sole determinants of their activities, but that binding sites may also play significant roles. Schepartz and coworkers arrived at a similar conclusion through their studies on the miniprotein activators [11]. Together these efforts pave the way to the development of potent activators by systematically analyzing and targeting protein surfaces found in the transcriptional complex.

In this issue of *Chemistry & Biology*, Mapp and coworkers show that the activity of the isolated peptide-ADs is sensitive to the nature and location of the DBD and to the presentation of the ADs on the DBD. These experiments raise a number of intriguing questions re-

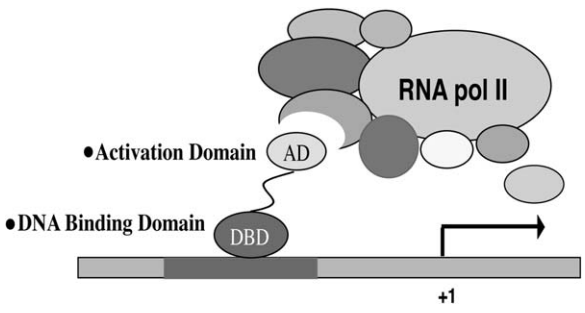

Figure 1. Activation of Gene Transcription

A minimal artificial activator composed of two separate functional domains: a DNA binding domain (DBD) and an activation domain (AD).

garding the location of the DBD relative to the initiation site and its effect on transcription activation. Significantly, the authors show that the artificial activators are only functional in the cells that contain the target protein. This level of specificity is uncommon in natural activators and constitutes a promising advance in the field [17].

The work summarized above describes continuing fundamental advances at the interface of chemistry and biology toward artificial control of gene expression. The latest addition by Mapp and colleagues provides a concrete foundation for designing a new generation of cell-type-specific and species-specific artificial activators. Certainly, much remains to be elucidated, because the ultimate goal is to generate cell-permeable transcription factors that not only turn gene transcription on or off but respond to extracellular signals as part of signal transduction cascades [3]. However, we can anticipate that chemists will continue to bring fresh perspectives and a zest for understanding biology at the molecular level to this highly fertile ground for exciting research.

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Selected Reading

1. Majmudar, C.Y., Lum, J.K., Prasov, L., and Mapp, A.K. (2005). Chem. Biol. *12*, this issue, 313–321.
2. Ptashne, M., and Gann, A. (2002). Genes and signals (New York: Cold Spring Harbor Laboratory Press).
3. Ansari, A.Z., and Mapp, A.K. (2002). Curr. Opin. Chem. Biol. *6*, 765–772.
4. Dervan, P.B., and Edelson, B.S. (2003). Curr. Opin. Struct. Biol. *13*, 284–299.
5. Nielsen, P.E. (2001). Curr. Opin. Biotechnol. *12*, 16–20.
6. Francois, J.C., Lacoste, J., Lacroix, L., and Mergny, J.L. (2000). Methods Enzymol. *313*, 74–95.
7. Segal, D.J., and Barbas, C.F. (2000). Curr. Opin. Chem. Biol. *4*, 34–39.
8. Frangioni, J.V., LaRiccia, L.M., Cantley, L.C., and Montminy, M.R. (2000). Nat. Biotechnol. *18*, 1080–1085.
9. Han, Y., and Kodadek, T. (2000). J. Biol. Chem. *275*, 14979–14984.
10. Rutledge, S.E., Volkman, H.M., and Schepartz, A. (2003). J. Am. Chem. Soc. *125*, 14336–14347.
11. Volkman, H.M., Rutledge, S.E., and Schepartz, A. (2005). J. Am. Chem. Soc. *127*, 000–000. in press. Published online March 4, 2005.. 10.1021/ja042761y
12. Asada, S., Choi, Y., and Uesugi, M. (2003). J. Am. Chem. Soc. *125*, 4992–4993.
13. Shimogawa, H., Kwon, Y., Mao, Q., Kawazoe, Y., Choi, Y., Asada, S., Kigoshi, H., and Uesugi, M. (2004). J. Am. Chem. Soc. *126*, 3461–3471.
14. Kwon, Y., Arndt, H.D., Mao, Q., Choi, Y., Kawazoe, Y., Dervan, P.B., and Uesugi, M. (2004). J. Am. Chem. Soc. *126*, 15940–15941.
15. Minter, A.R., Brennan, B.B., and Mapp, A.K. (2004). J. Am. Chem. Soc. *126*, 10504–10505.
16. Wu, Z., Belanger, G., Brennan, B.B., Lum, J.K., Minter, A.R., Rowe, S.P., Plachetka, A., Majmudar, C.Y., and Mapp, A.K. (2003). J. Am. Chem. Soc. *125*, 12390–12391.
17. Lu, Z., Ansari, A.Z., Lu, X., Ogirala, A., and Ptashne, M. (2002). Proc. Natl. Acad. Sci. USA *99*, 8591–8596.